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CELL CHANGES IN THE EPIDERMIS DURING THE EARLY STAGES OF REGENERATION IN THE TAIL OF THE FROG TADPOLE, WITH SPECIAL REFERENCE TO THE NUCLEUS-PLASMA RELATION[1]

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CONTENTS

I. STATEMENT OF THE PROBLEM

The following paper gives the results of a histological study of the cell changes in the epidermis during the early stages of regeneration in the tail of the frog tadpole, Rana clamitans. It deals with the migration of the epidermis which follows the operation and the accompanying behavior of the epidermal cells, with special reference to the nucleus-plasma relation.

[1]Contribution from the Zoological Laboratory of the University of Illinois, No. 47.

Fraisse (85), and Barfurth (90) both worked on the reparation of the epidermis and other epithelia in vertebrates, and decided that in the adult amphibian tail the new epidermis arises from the elements of the old epidermis, although no evidences of mitosis were found until the wound surface was completely covered with new epidermis.

Rand (01) found that also in the earthworm there is an epidermal investment of the wound surface, and in a later paper (04) decided that the new epidermis arises by means of a migration of the old epidermal cells, and described to some extent a few of the cell changes which accompany the migration, as does Emmel (10) for the crayfish.

Both of the last mentioned authors speak of a cytoplasmic decrease or an increase in nuclear volume of the cells in the migrating epidermis, either of which would change the nucleus-plasma relation.

The amphibian tail was selected because of the size and character of its cells, in that they are large and easily studied under the microscope. The present paper aims to give an account of the migration of the epidermis to cover the wound, and to describe the cell and nuclear changes in the migrating cells.

II. MATERIAL AND METHODS

Two dozen tadpoles of Rana clamitans averaging 75mm. in length were obtained in September, 1914, kept in an aquarium and fed on saltine crackers while awaiting experimentation. One fifth of the tail was removed and the stump allowed to regenerate for the desired time. When a portion of the regenerated tail had been cut off for study, the tadpole was put back into the aquarium and allowed to regenerate until the process stopped, and then used over again. This method has its advantages, as a section from a regenerated tail is much more easily cut than is the original tail, and makes thinner sections possible because of the softness of the tissues. By a comparison of sections from a regeneration of the original tail, and those from a tail previously regenerated, no difference was found.

Pieces were taken from tails of 5 minutes, 1 hour, 3 hours, 12 hours, 20 and 24 hours regeneration, fixed in Bouin's fluid, and imbedded in paraffin. A number of fixing fluids were tried, and by far the best results were obtained with Bouin. Sections were made 5μ in thickness, mounted in serial fashion, and stained with a number of different stains. Iron alum hæmatoxylin gave the best results for the determination of mitoses, while Ehrlich's acid hæmatoxylin and eosin furnished the best pictures for general tissue differentiation, as cell boundaries were well brought out.

III. OBSERVATIONS

1. *The behavior of the epidermis as a whole.*

The normal epidermis in the tail region of the frog tadpole is composed of several easily recognizable regions. At the surface I have not been able to make out a cuticle. The outer layer is composed of a definite layer of cells, cuboidal in section, with almost spherical nuclei (Fig. 6A). The cytoplasm at the surface stains slightly darker than that deeper in the cell (Fig. 6E). Next there are from one to five layers of cells with no regular arrangement (Fig. 6B), and a bottom layer of slightly columnar cells (Fig. 6C) with oval or round nuclei which have their long axis, if any, at right angles to the surface of the epidermis. Underneath these cells is a rather thick basal membrane, smooth staining, without fibrilation or striation of any kind (Fig. 6D). This membrane, which is drawn dead black in all of the illustrations, is of great importance in the present study, as it is not regenerated in the early stages, and therefore the place where the epidermis has been cut can be easily seen by determining where this membrane stops.

A number of experiments were tried in order to watch the course of the regeneration in the living material under the microscope, but without material success.

Figures 1-5 show a series of sections near or through the notochord at various times of regeneration, drawn semi-diagrammatically to show the action of the epidermis. Fig. 1 is a frontal section of a tail of a 1 hour regeneration. This shows very clearly the first process which takes place in the regeneration. The muscles have contracted both inward and around the tail, thus decreasing

to some extent the wound surface. This contraction has the effect of rounding over the square edges of the cut end, and bends the epidermis around on the lateral sides so that the epidermis of the opposite sides touch near the dorsal and ventral sides of the tail where it is very narrow. At these places the epidermis fuses, and thus further decreases the wound area. The epidermis has not at this time begun to move, which is well brought out by the fact that the basal membrane has about the same relation to the epidermis as it had at the time of the cut (Fig. 1B). A clot is being formed to cover the wound, in which black staining fragmenting nuclei of

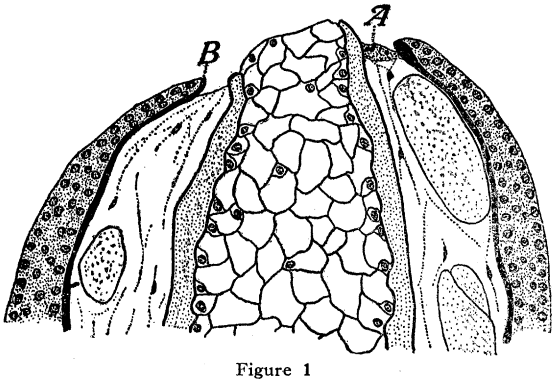


Figure 1

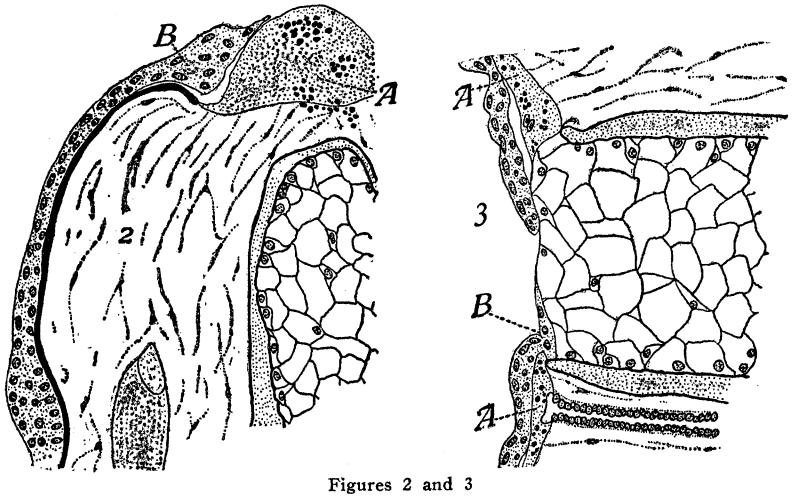
Fig. 1. Frontal section through the notochord 1 hour after the operation, showing the relation of the epidermis to the basal membrane, and the beginning of the formation of the blood clot. A, blood clot; B, basal membrane. (200 diameters)

degenerating cells are beginning to be seen. In most cases the next thing to appear is a complete blood clot over the wound surface, although the time of the formation may be before one hour has elapsed, or later, but in all cases the clot was formed to a greater or less degree before 12 hours had past (A-Figs. 1-5).

Figures 2 and 3 show the period of greatest activity in the epidermis. Figure 2 is a frontal section, and Figure 3 is a sagittal section of a regeneration of 12 hours duration. As will be seen in Figure 2 the epidermis has left the basal membrane behind, and has commenced on its advance over the wound tissues and the clot (Fig. 2B). This plainly shows the importance of having some landmark by means of which one may tell whether or not there has

been an actual advance of the epidermis, or whether this apparent advance is only a flap of epidermis left by an incomplete cut which has folded over the wound surface. In the several cases in which the latter happened, it was immediately noticed because of the accompanying basal membrane. In the normal epidermis the cells seem to stick rather closely to this membrane, and in the normal individual when the epidermis is torn off, the basal membrane usually comes away with it.

Figure 2A and 3A show the position of the blood clot over the wound surface. In the tail from which Figure 2 was taken, the clot covers the entire cut surface including the notochord, while in Figure 3, the notochord is not covered. This is a very finely fibrinated clot with a rather thicker fibranation near its outer surface. It is for the most part homogeneous, however, and has in its numerous fragments of the nuclei of the degenerating cells. These fragments arise by a process of amitosis, or fragmentation, as it perhaps had better be called. (Sutherland-15.) At first these



Figures 2 and 3

Fig. 2. Frontal section near the notochord 12 hours after the operation, showing the advancing end of the epidermis, and the blood clot covering the entire wound. A, blood clot; B, advincing end of epidermis. (200 diameters)

Fig. 3. Sagittal section through the notochord 12 hours after the operation, showing the advincing edges of the epidermis and the blood clots on each side of the notochord. A, blood clot; B, advancing end of epidermis. (200 diameters)

fragments do not stain very dark, and seem to come from the cells near the cut surface, but later they stain almost black, and for some distance back into the tissue immediately behind the cut edge the nuclei take the heavy black stain, showing, in all probability, a degenerative process. One very peculiar thing was found during the early stages, in that while all of the muscle, connective tissue, notochordal, and nerve cord cells for some distance back from the cut show fragmentation and degeneration, not a single nucleus of the epidermis was found to have a degenerative appearance, as evidenced by fragmentation and deep staining.

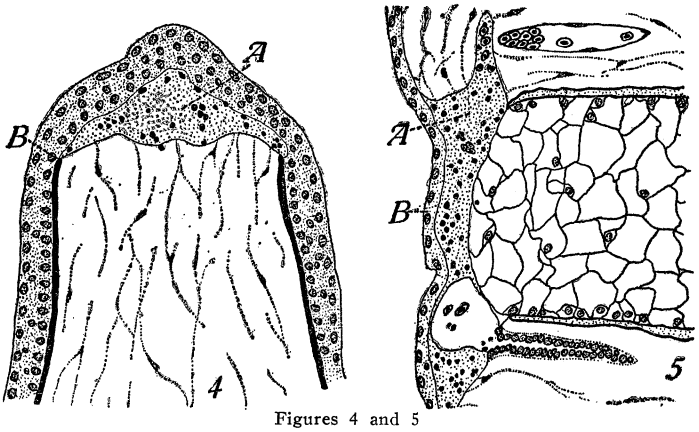
In the early migration the cells seem to travel in two different ways. In Figure 2, the advancing end of the epidermis is composed of several different layers of cells (Fig. 2B), while the advancing edge of the epidermis in Figure 3 is composed of only one layer of cells (Fig. 3B). It appears to me that all of the cell layers would advance nearly equally as in Figure 2 if the configuration of the surface ahead of it would allow it to do so. The surface in Figure 3 is somewhat obstructed, and as it is the outer layer of cells which is the most active in the migration, the cells below are stopped, and the outer layer passes over the obstruction.

Figures 4 and 5 show a frontal and a sagittal section of a regeneration of twenty hours. Figure 4 shows that the epidermis has advanced over the clot and met the epidermis from the other side and fused with it. It also shows that the basal membrane is in the same position as it was when cut (Fig. 4B). The epidermis here which covers the clot is of normal thickness, and the cells seem to have returned to their normal position and shape. Figure 5 does not seem to be as far along as Figure 4 as in this case the investment of epidermis is only a single cell thick. This is the result of an obstruction of the deeper layers of the epidermis as mentioned above, and the investment will not reform its several layers until the beginning of mitosis at about 48 hours.

One of the first things which is noticed in the movement of the epidermis is the lack of any evidences of cell multiplication in the epidermis, either near the point of movement, or farther back, where mitosis or an increase of cells by any means would aid in pushing the epidermis over the wound. There seem to be even

fewer mitoses than normally in the epidermis. In all of the sections which have been examined up to a stage of 20 hours regeneration there was only one mitotic figure found. This was near the region where the epidermis was normal, and had no part in the movement of the tissue.

I will now sum up briefly the movement of the epidermis as a whole. As a result of a strong muscle contraction of the tail during the first hour, the epidermis from the two sides is brought in contact above and below the notochord, thus decreasing the wound



Figures 4 and 5

Fig. 4. Frontal section just above the notochord 20 hours after the operation, showing the wound healed by the meeting of the epidermis from the two sides, enclosing the blood clot. A, blood clot; B, basal membrane, marking position of cut. (200 diameters)

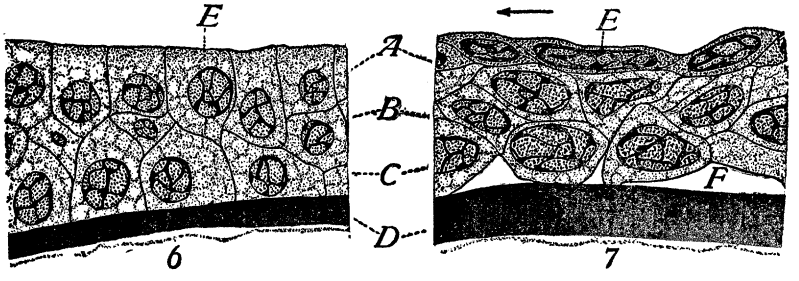
Fig. 5. Sagittal section through the notochord 20 hours after the operation, showing wound healed by meeting of epidermis from the two sides, enclosing the blood clot. Note that here the epidermis is only one cell in thickness, while in Fig. 4 the investment is several cells in thickness. A, blood clot; B, epidermal investment. (200 diameters)

area. The epidermis at these places fuses and plays no further part in the investment of the cut surface. The epidermis surrounding the cut notochord and the thick part of the tail then starts to migrate over the blood clot and wound surface, unhelped by cell multiplication within the tissue. The epidermis from the two sides meets near the center of the cut surface, and the cells return to their normal shape and size. If the epidermis has been able to migrate with all layers of cells, normal stratification is resumed. If only

a single layer of cells has migrated over the wound surface the epidermis remains a single cell in thickness until mitosis begins. This action encloses the blood clot which has been formed and is complete at from 20 to 24 hours. The next process which occurs is the absorption of the blood clot and when this is completed, at about 48 hours, regeneration by mitosis begins.

2. The behavior of the individual cells in the epidermis.

As has been stated, the normal epidermis is composed of several definite layers, the most conspicuous of which are the upper layer of cuboidal cells, and the lower layer of columnar cells. There are several layers of cells between these two limiting layers.



Figures 6 and 7

Fig. 6. Section through the normal epidermis showing the relation of the layers of cells. A, outer layer; B, intermediate layer; C, columnar layer; D, basal membrane; E, darker cytoplasm at surface. (1100 diameters)

Fig. 7. Section through the migrating epidermis some distance away from the advancing end, showing the change in shape of cell and nucleus. A, outer layer; B, intermediate layer; C, columnar layer pulling away from basal membrane; D, basal membrane; E, greatly elongated cell at surface; F, space between basal membrane and epidermis. (1500 diameters)

The lower columnar layer is tightly stuck to the basal membrane. In the twelve hour stage of regeneration, which seems to be the stage in which the migratory movement is the greatest, as we leave the normal epidermis and approach the cut edge, the first noticeable thing is that the columnar cells on the basal membrane lean over in the direction of the cut surface, so that their nuclei instead of being at an angle of 90° with the basal membrane, are coming to be more nearly parallel with it (Fig. 7C). At the same time the upper layer of cuboidal cells commences to lose its stratification,

and the cells change from a cuboidal shape to one which is elongated in the direction of the cut surface (Fig. 7E). In following along the epidermis of a 12 hour preparation, one can find all stages in the change of shape, from the cuboidal to the greatly elongated cell at the place of greatest movement.

Immediately after the basal columnar cells start to lean in the direction of the cut surface, spaces appear between the basal cells and the basal membrane, so that finally the basal cells are in connection with the basal membrane in only a few places (Fig. 7F). These spaces are large and do not appear to be in the cytoplasm of the basal cells, but rather in the region between the cell and the membrane. The nuclei of the basal cells do not appear to change

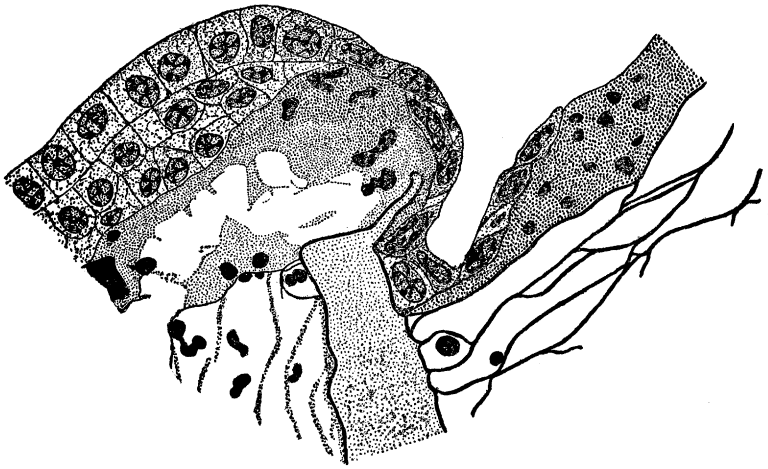


Figure 8

Fig. 8. Section through the notochord, showing the advancing end of the epidermis composed of a single layer of cells. (900 diameters)

their shape to any great degree, merely rotating through 90° so that their major axis is parallel to the direction of movement. This is not so, however, with the cells of the upper layer. Here the nuclei in the normal epidermis were nearly spherical, so that when the cell elongates in the direction of movement, the nuclei are squeezed into an elongated shape.

After the cells have passed the place where the cut was made, most of their nuclei except those of the upper layer have resumed

their normal spherical or slightly elongated shape. Those of the upper layer are still in an extremely elongated condition, as are the cells. In some of the cases found, it was only this upper layer which was migrating over the clot surface (Fig. 8). This would seem to show that the upper layer of cells was the one which was doing the most active migration, and that they were pulling along the lower cells. As was mentioned before it is this outer layer which passes around obstructing portions of the wound surface.

In fact, it is only in this external layer of cells that any change in nuclear shape and change in chromatin arrangement can be detected. The nucleus changes from a spherical to a greatly elongated shape. This is due, I am convinced, not to any action of the nucleus itself, but is merely a passive adjustment to conditions laid upon it by the moving cell. However, there does seem to be a change of some kind, for the nucleus takes on a rather deeper stain than it did in the normal epidermis, and the chromatin does not appear to be arranged in exactly the same manner as normally. In the normal nucleus of the outermost cells, the chromatin is in a rather coarse reticulum, differing in no way from that of any of the other cells in the epidermis. After migration starts the chromatin appears to be in a much coarser network than formally, with thick strands of chromatin parallel to the direction of movement. I do not find any evidences of "polarization" as does Emmel (10) in the regeneration of the epidermis in the crayfish. The appearance of these cells under the microscope is one of movement, as they extend their cytoplasm forward toward the cut surface in a pseudopodial manner. In no case did I find any cells positively identifiable with epidermal cells wandering loose. Several isolated cells were found, but were decided to be leucocytes.

After the clot has been covered, whether it be by one or more layers of cells, these outside cells regain their normal shape, and the nuclei come back to the spherical form. Their migratory movement is finished with the fusion of the epidermis of the two sides of the tail. After this fusion and the resumption of the normal shape, there is a resting period of about 24 hours while the clot is being absorbed. During this latter period there is no mitosis, and the epidermal cells have every appearance of normal epidermal

cells, altho as has been said there may be only one layer. In this connection it may be said that a count was made to determine whether or not there is amitosis in the regenerating epidermis after mitosis has begun, and the resulting ratio was about 100 characteristic mitotic figures to one dumbbell shaped nucleus, which may or may not be an evidence of amitosis. In fact, after a careful study of the later stages of the regeneration, I am inclined to think that amitosis plays no part in the process. Of course, it is hard

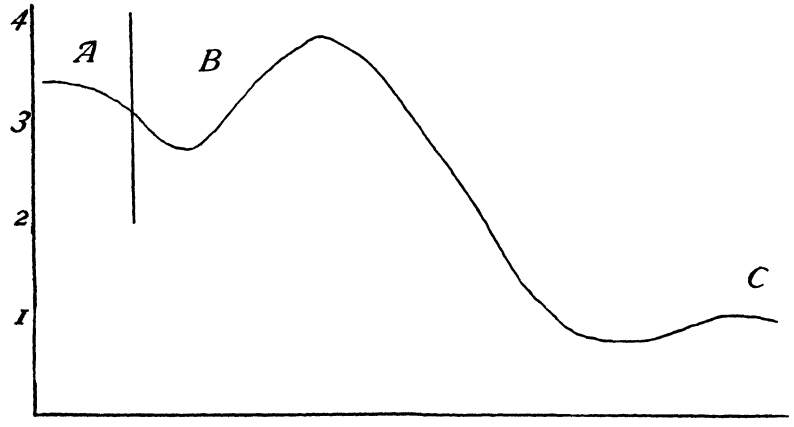


Figure 9

Fig. 9. Curve showing the decrease in the nucleus-plasma relation in the migrating cells, taken from a frontal section. The abscissa represents the position of the cell in the epidermis, and the corresponding ordinate the nucleus-plasma ratio for the cell. A, region of normal epidermis; B, region of the beginning of migration; C, region of the advancing end and active migration.

to recognize true amitotic figures, but from all the observations made there was not the slightest evidence that amitosis was characteristic or even common in the regenerating epidermis.

3. The nucleus-plasma relation in the migrating epidermis.

The nucleus-cytoplasm ratio was obtained by a method described by Dolly (13) in which the measurements for the axes of the cells and nuclei are made from camera lucida drawings at a magnification of about 1000, with a rule graduated to 1mm. The relative volumes are calculated by supposing that the unknown third dimension is equal to the shortest axis as found in a single

section. These three dimensions are then multiplied together, subtracting the nuclear volume from that of the cell. The resulting figure for the plasma mass when divided by the nuclear volume gives a figure corresponding to Richard Hertwig's conception of the nucleus-plasma ration.

A glance at Figure 9 will show at once some of the results. The curve was obtained by plotting the cell measurements in the relative positions the cells held in the migrating epidermis between the normal portion and the advancing end.

As will be seen from the curve, the nucleus-plasma ratio of the cells in the normal epidermis is about 3.5. The ratio seems to increase slightly as the cells begin to leave their regular positions in the epidermis, and then to steadily decrease until the ratio in the actively migrating cells is about 1.0. These ratios were obtained from a frontal section.

This reduction is extremely interesting if the results of such a measurement can be relied upon. Of course it is not necessary to have an accurate measurement of volume, it being only important that the calculations apply to all of the cells without a variation due to the changes in the shape of the cells. It may be interesting to note in this connection that the change in the nucleus-plasma relation takes place along with the change in shape of the cell. This might account for the difference in ratio, but I am inclined to think that there is in reality a change in the nucleus-plasma relation, for this method will give the volume of a parallelopiped whether it be a cube or otherwise.

Also, to check the results obtained in Figure 9, which was made from a frontal section, measurements were made from a sagittal section of a tail at the same time of regeneration. If the entire cell merely spread out, then the section through this plane would certainly not show a decrease in the nucleus-plasma relation, but rather an increase to compensate for the decrease obtained from a frontal section. This, however, is not so. A curve from a measurement of a sagittal section (Fig. 10) shows essentially the same characteristics as the curve in Figure 9. These two curves combine all three dimensions of the migrating cells, whereas a curve from a sagittal or a frontal section alone, will have an unknown

dimension which has to be estimated. In this way, by measuring and making curves from frontal and sagittal sections, I think I have overcome the objection which might be made because of the estimation of the third or the unknown dimension. The proof of this is that both curves show a decrease in the ratio as they should if there were a true decrease in the cells.

IV. DISCUSSION

As will be evident from the foregoing, the epidermis which covers the wound and the clot comes, without a doubt, from the old epidermis. There is a slight difference in the regeneration in

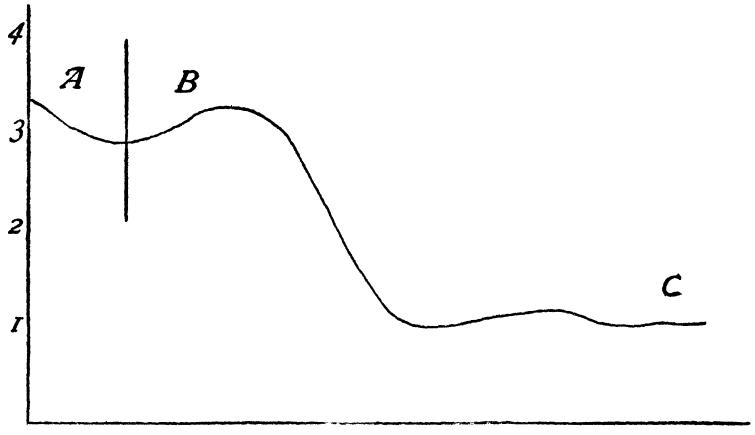


Figure 10

Fig. 10. Curve showing the decrease in the nucleus-plasma relation in the migrating cells, taken from a sagittal section.The abscissa represents the position of the cell in the epidermis, and the corresponding ordinate the nucleus-plasma ratio for the cell. A, region of normal epidermis; B, region of the beginning of migration; C, region of the advancing end and active migration.

this form from that described by Rand (04) for the earthworm, in that he says "as already pointed out, mere contraction of the muscular body wall plays an unimportant part in the closing of the wound, because of the relatively large cross section of the worm." In the tadpole the wound is very narrow except at the region of the notochord, and in one hour's time the contraction of the muscles of the tail is sufficient to cause the meeting of the

epidermis of the two sides above and below the notochord it was seen that the basal membrane, and therefore the epidermis, even overlaps to a slight degree. So it may be said that the first reparation process, that of muscle contration is of some importance in the early stages of regeneration in this form. There cannot be any doubt that the new epidermis comes from the old because it may be seen in various stages on its way, and because there is no tissue with which it might be confused which has anything like the appearance of the epidermis.

Rand (04) and Emmel (10) differ somewhat as to the internal cell changes. Rand states for the earthworm "the nuclei, however, never show marked change in volume" while Emmel says that in the crustacean "it may be noticed that the nuclei are becoming larger in size, and the chromatin is undergoing certain changes." I could find no evidence in either direction in the tadpole, migrating nuclei being in some cases both larger and smaller than normal nuclei. Rand does not mention any marked chromatin changes, while Emmel describes a kind of "polarization" of the chromatin in the nucleus. I did not find any radical changes in the chromatin except that it seemed to accumulate in larger bodies than normally, and that it was more densely packed, due perhaps to the lengthening of the nucleus.

There can be no doubt that in the tadpole there is a migration of the individual cells of the epidermis. The extraordinary change of shape of the cells, especially those of the outermost layer indicates that the individual cell is moving by lengthening out in an amœboid manner. This migration cannot be otherwise, for if there was a pulling of all the epidermis from the region of the cut surface, all of the cells would take on the elongated form so characteristic of the outermost layer of cells. This, however, is not so, and it is this same outer layer which is the most changed in shape, and which seems to be the most concerned in the process of migration. The deeper cells have the appearance of being pulled along, and to support this hypothesis, instances have been noted, where the deeper layers of cells are obstructed by some of the fragments of the cut tissues, and have been held back, only the outer layer traveling over the obstruction.

The advancing edge of the migrating epidermis clearly shows then that there is not a passive pushing of the cells over the cut surface. What is the agency by which the cells are stimulated to move over the wound? They always move over the surface. In no case did I find evidences of a migration down into the clot, nor were there any signs of a free migration, that is, without a solid support for the migrating cells. Neither were the advancing cells moving along the fibrils of the clot, as they moved as easily over the cut notochord. The fibrinations of the clot also were too small to give anything more than a rough surface over which the cells might travel. There was nothing like the condition of the spider web which was used by Harrison (14) in his experiments with cell migration. The migration here is more like that shown by the cells which moved along the cover glass in his experiments.

This migration is assigned by the majority of authors to chemotaxis, or a chemotactic cell phenomenon, in the sense of Roux (94) (96). The stimulus may come from the chemical attraction of the wound surface, or the mutual interaction of the epidermal cells. The stimulus starts the epidermal cells moving and as the only direction in which they can move is over the wound surface the effect of their migration is to heal the wound opening. When the two sides meet there is no longer any opportunity for migration as there is no place for the cells to go, so that migration stops. Rand emphasizes the fact that the stimuli operating there are similar in nature to those which are said by Herbst (94) to be of great importance in normal ontogeny.

There have been several references to a change in the size of the cell or nucleus during the migration of the epidermis in various animals. Rand (04) mentions the fact that the cytoplasm of the migrating cells in the earthworm appears to decrease, and Emmel (10) says for the crayfish that the nuclei seem to be slightly larger than normally. Fraisse (85) states that on the advancing edge of the migrating epidermis of the salamander the nucleus is surrounded by a small amount of protoplasm, so that the nuclei are closely packed together. A glance at his Plate I, Fig. 8, shows this feature, and the change in relation of the cytoplasm to the nucleus is plainly to be seen without measurement.

It seems fairly certain, therefore, that there is a decrease in the nucleus-plasma ration during migration. The measurements given in this paper of course are not useful as actual numerical values of the volumes, being merely to get an approximate curve of the decrease in the ratio value.

Admitting the fact that there is a decrease in the nucleus-plasma ratio, what does it signify? If Minot's hypothesis applies to all forms of cell phenomena in which there is a decrease in the nucleus-plasma ratio, then this might indicate a rejuvenescence of the cell after differentiation. He states "Rejuvenescence depends on the increase of the nucleus" or in terms of the nucleus-plasma relation a decrease in that ratio. This was in connection with cell cleavage of the fertilized egg. He also adds "Reversed cytomorphosis is not known to occur, or in other words differentiated material cannot be restored to the undifferentiated condition." Yet here if the decrease in the nucleus-plasma ratio indicates a rejuvenescence we have differentiated epidermal cells which are rejuvenating and afterward returning to the differentiated condition.

Interesting in this connection is the work of Morgulis (11) on inanition, in which he describes the effect of starvation on the cells of the salamander, Diemycytylis viridescens. He concludes that the volume of both the cell and nucleus decreases as a result of starvation, but that the rate of diminution of the volume of the cells is greater than that of the nuclei.

The basal cells of the epidermis in the tadpole pull away from the basal membrane during migration, and it is possible that this decreases the interchange of nutrient material between the blood and the epidermal cells. Therefore they may be reduced to the condition of starved cells. This, if Morgulis' hypothesis be correct would tend to reduce the nucleus-plasma ratio. Therefore the decrease in the observed nucleus plasma relation may be entirely due to a starvation of the epidermal cells caused by isolation from food during migration.

However, Conklin (12) states that rejuvenescence is dependent on the interchange of material between the nucleus and the cyto-

plasm, and that anything which facilitates this interchange increases metabolism and leads to rejuvenescence.

The cells are in active motion, and as the supply of food may be cut off, and as they are undergoing an unusual activity the rate of metabolism is without doubt great. This activity is so great that the cytoplasm is probably used up and therefore the nucleus-plasma ratio decreases. Therefore, if Conklin's hypothesis applies to these cells we have a rejuvenescence as there is a high rate of metabolism and consequently an interchange of material between nucleus and cytoplasm.

V. SUMMARY.

In brief the processes which occur in the early stages of regeneration in the tail of Rana clamitans are as follows:

1. There is a muscle contraction within 1 hour after the cut which not only decreases the surface of the wound, but also plays an important part in the process of healing by bringing the epidermis of the two sides of the tail together both above and below the notochord, where the epidermis fuses.

2. There is a migration of the epidermal cells over the clot which is formed at about 12 hours time. This migration is the result of an active migration of the individual cells of the epidermis, especially those of the outer layer, without the slightest sign of cell multiplication of any sort, either by mitotic or amitotic cell division.

3. There is a decrease in the nucleus-plasma ratio in the migrating epidermis.

4. As the cells of the migrating epidermis are pulled away from their normal position in relation to the blood vessels, this decrease may be due to starvation.

5. As there is also a very active migration of the individual cells the rate of metabolism is probably high, and as the cells may not receive their customary amount of nourishment, the cells may be forced to rely upon the energy contained in the cytoplasm, and so use it up during the migration. This would account for the decrease in ratio.

6. If the views of Minot and Conklin can be applied to these cells, then this decrease in the nucleus-plasma ratio would indicate a rejuvenescence of differentiated epidermal cells.

7. Migration ceases at about 24 hours and the nucleus-plasma ratio returns to normal in the epidermal cells.

8. The period of time from 24 to 48 hours is occupied by the absorption of the clot, and the cells are in a resting condition.

9. At 48 hours regeneration by mitosis begins. No evidence has been found that amitosis is a factor in the regeneration of the tail of the frog tadpole.

This problem was suggested by Dr. Charles Zeleny, to whom I am indebted for many helpful suggestions during the course of the investigation.

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